TZU CHI MED J June 2008 Vol 20 No 2

available at http://ajws.elsevier.com/tcmj

Tzu Chi Medical Journal

Review Article

Cardiovascular Actions of Endomorphin-2 in the Nucleus Tractus Solitarius

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Article info

Article history:
Received: February 1, 2008
Revised: February 11, 2008
Accepted: March 11, 2008

Keywords:
Baroreflex
Blood pressure
Bradycardia
Depressor responses

Abstract

Cardiovascular effects of microinjections of endomorphin-2 into the medial subnucleus of the nucleus tractus solitarius (mNTS) were studied in adult, male, urethane-anesthetized, Wistar rats. Microinjections of endomorphin-2 into the mNTS elicited depressor and bradycardic responses via mu-opioid receptors. Similar microinjections of endomorphin-2 attenuated the carotid sinus and aortic baroreflex responses. Endomorphin-2 inhibits GABAergic mNTS neurons via mu-opioid receptors, causing disinhibition which results in excitation of secondary mNTS neurons. This effect causes depressor and bradycardic responses. Endomorphin-2 also inhibits glutamate release from baroreceptor terminals via mu-opioid receptors located on these terminals and attenuates baroreflex responses. (*Tzu Chi Med J* 2008;20(2): 77–81)

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1. Introduction

The role of the medial subnucleus of the nucleus tractus solitarius (mNTS) in cardiovascular regulation is well established (1,2). Peripheral afferents (e.g., baroreceptor, cardiopulmonary and chemoreceptor afferents) are known to make their primary synapse in this subnucleus. Although there is a general consensus that glutamate is the neurotransmitter for regulating cardiovascular function in the mNTS (2,3), the presence of several other substances has been demonstrated in this region. Among these substances are endomorphins (two tetrapeptides, endomorphin-1 and -2) that were isolated from human and bovine brains (4,5). These peptides have been reported to possess a high affinity and selectivity for the mu-opioid receptors and are considered to be endogenous ligands for these receptors (4,5). The presence of mu-opioid receptors (6–8) and endomorphins (9,10) in the mNTS has been reported. These reports have prompted investigations on the role of endomorphins in the mNTS in cardiovascular regulation (11,12).

2. Cardiovascular responses to endomorphin in the mNTS

In urethane-anesthetized (1.2–1.4 g/kg, i.v.), artificially ventilated, adult, male Wistar rats, microinjections of L-glutamate (L-Glu) into the mNTS (0.5–0.6 mm rostral and 0.5–0.6 mm lateral to the calamus scriptorius and 0.5–0.6 mm ventral to the dorsal surface of the medulla) were used to identify mNTS in all experiments. L-Glu (5 mmol/L) elicited decreases in mean

arterial pressure (MAP; 35–45 mmHg) and heart rate (HR; 30–40 beats/min); L-Glu stimulates neuronal cell bodies but not fibers of passage indicating that the cardiovascular responses were mediated by neurons in the mNTS. A 100 nL volume elicited maximum responses; this volume was selected for all microinjections unless indicated otherwise. Microinjections of endomorphin-2 (0.1, 0.2, 0.5, 1, 2 and 4 mmol/L) into the mNTS (n=30) elicited decreases in MAP (25±5.3, 40±2.8, 30±3.1, 16.6±4.5, 18±3.4, and 15±1.5 mmHg, respectively) and HR (20±3.2, 50±7.0, 37.5±10.3, 33.3±8.8, 20±10.0, and 15±2.9 beats/min, respectively). Maximal depressor and bradycardic responses were elicited by a 0.2 mmol/L concentration. The onset and durations of the responses to microinjections of endomorphin-2 (0.2 mmol/L) were 5–10 seconds and 8–10 minutes, respectively, and the peak effect was observed at 2–3 minutes. When the concentrations of endomorphin-2 that elicited depressor and bradycardic responses in the mNTS (e.g., 0.2 mmol/L) were injected intravenously, no responses were elicited, indicating that the leakage of endomorphin-2 from the microinjection site to the peripheral circulation was not responsible for the observed responses. Repeated microinjections of endomorphin-2 (0.2 mmol/L) into the mNTS did not exhibit tachyphylaxis when the interval between injections was at least 20 minutes. The possibility that the cardiovascular responses elicited by microinjections of endomorphin-2 into the mNTS may have resulted due to the spread of the injected peptide to adjacent regions was excluded by microinjecting the peptide into sites adjacent to this nucleus (e.g., cuneate nucleus); endomorphin-2 did not elicit a response at these sites. Microinjections (100 nL) of artificial cerebrospinal fluid (aCSF) into the mNTS did not elicit any response, indicating that local distortion of brain tissue was not responsible for the endomorphin-2 induced responses [12].

3. Effect of vagotomy on endomorphin-induced bradycardia

Bilateral vagotomy abolished the bradycardia elicited by microinjections of endomorphin-2 (0.2 mmol/L) into the mNTS while the decreases in MAP remained unaltered, indicating that the bradycardic responses to microinjections of endomorphin-2 into the mNTS were mediated predominantly via the vagus nerves [12].

4. Role of mu-opioid receptors in mediating endomorphin responses

Naloxonazine (1 mmol/L) was used to demonstrate that the responses to endomorphin-2 were mediated via mu-1 opioid receptors [13]. Microinjection of endomorphin-2 (0.2 mmol/L) into the mNTS elicited decreases in MAP and HR. Twenty minutes after recovery of the responses, naloxonazine (1 mmol/L) was microinjected at the same site. Microinjection of naloxonazine alone elicited no cardiovascular responses. Two minutes after the microinjection of naloxonazine, microinjection of endomorphin-2 failed to elicit any response. The lack of response to endomorphin-2 was not due to tachyphylaxis because repeated microinjections of endomorphin-2 did not exhibit tachyphylaxis as described earlier. A lower concentration (0.5 mmol/L) of naloxonazine did not significantly block the responses to endomorphin-2 (0.2 mmol/L). That naloxonazine did not exert any deleterious effects at the site of injection was indicated by the observation that this antagonist did not alter the depressor and bradycardic responses to microinjections of L-Glu (5 mmol/L) injected 2 minutes after endomorphin-2 [12].

5. Effect of GABA receptor blockade on endomorphin-induced responses

In these experiments, microinjections of endomorphin-2 (0.2 mmol/L) into the mNTS elicited usual depressor and bradycardic responses. These responses were abolished by prior combined microinjections of gabazine (2 mmol/L; $GABA_A$ receptor antagonist) and 2-hydroxysaclofen (100 mmol/L; $GABA_B$ receptor antagonist). Gabazine and 2-hydroxysaclofen did not alter the responses to microinjections of L-Glu into the mNTS [12].

6. Effect of glutamate receptor blockade on endomorphin-induced responses

The depressor and bradycardic responses to microinjections of endomorphin-2 (0.2 mmol/L) into the mNTS were completely blocked by prior combined microinjections of NBQX (2 mmol/L; non-NMDA receptor antagonist) and DAP-7 (5 mmol/L; NMDA receptor antagonist). Combined microinjections of NBQX and D-AP7 did not alter the depressor and bradycardic responses to an unrelated agonist carbachol (0.5 mmol/L; cholinergic receptor agonist) [12].

7. Effect of endomorphin on single neurons

The basal firing rate of mNTS neurons, recorded extracellularly using glass micropipettes, was 15.9±2.2 spikes/sec. An intravenous bolus injection of phenylephrine (3 μg/kg) increased MAP (40±4.8 mmHg)

and increased neuronal firing in the mNTS, indicating that the neuron was involved in baroreflex and, therefore, cardiovascular regulation. When the MAP returned to basal level, L-Glu (5 mmol/L) was applied to the neurons; the ejection volume of all direct applications of different agents on neurons was 2–4 nL. Application of L-Glu increased the neuronal firing, indicating that the recording was from a neuron and not a fiber of passage. Application of aCSF did not elicit a response, indicating that pressure applications alone were not responsible for the changes in neuronal firing. Application of endomorphin-2 (0.2 mmol/L) increased the firing of the mNTS neurons. Application of naloxonazine (1 mmol/L) did not alter the neuronal firing, however, it blocked the excitatory effect of subsequent application of endomorphin-2. Naloxonazine did not alter the responses to direct application of L-Glu (5 mmol/L). In other neurons identified similarly, combined application of D-AP7 (5 mmol/L) and NBQX (2 mmol/L) decreased the basal firing rate. After the firing returned to basal level (40–50 sec), subsequent application of endomorphin-2 (0.2 mmol/L) failed to excite the neuron [12].

8. Endomorphin-induced attenuation of baroreflex responses

The carotid sinus was isolated from the general circulation and connected to a perfusion assembly. The pressure in the carotid sinus was increased and the systemic cardiovascular responses studied before and after the microinjections of endomorphin-2 (0.4 mmol/L) into the mNTS. Microinjections of endomorphin-2 into the mNTS attenuated the carotid sinus baroreflex responses. Recovery from the endomorphin-2-induced attenuation of carotid baroreflex was observed within 20 minutes [14].

In other experiments, the aortic nerve was electrically stimulated and cardiovascular responses studied before and after microinjection of endomorphin-2 (0.4 mmol/L) into the mNTS. Microinjections of endomorphin-2 into the mNTS attenuated the responses to aortic nerve stimulation. Recovery from endomorphin-2-induced attenuation of aortic baroreflex responses was observed within 20 minutes [14].

The role of endogenous opioid receptors in baroreflex responses was tested using aortic nerve stimulation. Naloxone (0.5 mmol/L) blocked the effects of endomorphin-2 (0.4 mmol/L) but not those of L-Glu (5 mmol/L), indicating that naloxone at this dose did not exert any deleterious effects in the mNTS. Lower concentrations of naloxone (e.g., 0.25 mmol/L) did not attenuate the cardiovascular responses to endomorphin-2 (0.4 mmol/L). Blockade of opioid receptors in the mNTS by naloxone alone did not alter cardiovascular responses to aortic nerve stimulation [14].

9. Conclusions

Microinjections of endomorphin-2 (0.1–4 mmol/L) into the mNTS elicited depressor and bradycardic responses. Endomorphin-induced bradycardia was mediated via the activation of the parasympathetic innervation to the heart because bilateral vagotomy completely abolished these responses. Both the depressor and bradycardic responses were mediated via mu-1 opioid receptors because naloxonazine, a specific antagonist for mu-1 opioid receptors [13], abolished them. Microinjections of naloxonazine by itself did not elicit any response, suggesting that mu-1 opioid receptors in the mNTS are not endogenously active in the rat.

The blockade of GABA receptors in the mNTS abolished the depressor and bradycardic responses to microinjections of endomorphin-2 into the mNTS. Endomorphin-induced depressor and bradycardic responses were also abolished after the blockade of ionotropic glutamate receptors in the mNTS.

Neuronal recording experiments confirmed our results obtained in the microinjection studies [12]. Excitatory effect of endomorphin-2 on the mNTS neurons was prevented by prior application of naloxonazine; naloxonazine by itself did not elicit any response. Endomorphin-2-induced excitation of the neurons was completely abolished by prior applications of ionotropic glutamate receptor antagonists (D-AP7 and NBQX).

Microinjections of endomorphin-2 into the mNTS attenuated the reflex responses (i.e., decrease in BP and HR) to the stimulation of carotid sinus and aortic baroreceptors [14]. The effects of endomorphin-2 were mediated via opioid receptors because prior microinjections of naloxone (an opioid receptor antagonist) prevented these responses. However, microinjections of naloxone alone did not alter the baroreflex responses, indicating that opioid receptors in the mNTS are not normally involved in regulating the baroreflex function.

The excitatory effect of microinjections of endomorphin-2 on mNTS neurons resulting in depressor and bradycardic responses on the one hand and the inhibitory effect of these microinjections on baroreflex on the other hand can be reconciled as follows. Immunohistochemical studies have demonstrated the presence of GABAergic neurons [15,16] and terminals [17] in the NTS. Glutamatergic nerve terminals of peripheral baroreceptor and cardiopulmonary afferents are also known to make their first synapse in the mNTS [1–3]. There is a general consensus that opioid peptides interact with G-protein coupled opioid receptors and usually exert inhibitory effects on neurons [18–21]. Thus, inhibition of GABAergic neurons by endomorphin-2 via mu-1 receptors may result in preponderance of glutamatergic input to the secondary mNTS neurons. This may be the reason why endomorphin-2-induced depressor and bradycardic

responses were no longer observed after GABA receptor blockade. Furthermore, blockade of ionotropic receptors also resulted in the abolition of endomorphin-2-induced depressor and bradycardic responses.

The attenuation of baroreflex responses by endomorphin-2 can be explained by either endomorphin-2-induced reduction in responses of postsynaptic mNTS neurons involved in baroreflex or inhibition of neurotransmitter release from the baroreceptor terminals. Reduction in the postsynaptic responses of the neurons involved in baroreflex does not seem to be the mechanism by which attenuation of baroreflex is elicited by endomorphin-2 because the responses to microinjections of NMDA, which acts predominantly postsynaptically (22), remained unaltered after microinjections of endomorphin-2 into the mNTS. Therefore, attenuation of baroreflex responses by endomorphin-2 may be ascribed to a reduction in the release of neurotransmitter from the baroreceptor terminals. Since glutamate has been reported to be the neurotransmitter released from baroreceptor terminals in the mNTS (3), endomorphin-2 may inhibit its release by a presynaptic action. In this context, it may be pointed out that opioid receptors have been reported to be present on presynaptic terminals of vagal afferents in the NTS (23) and opioid peptides have been shown to inhibit glutamate release from nerve terminals (8, 24–26). For this explanation to be true, opioid receptors must be present either exclusively or preponderantly on the glutamatergic baroreceptor terminals. Supporting this hypothesis is the report in which only 33% of the vagal afferents contained labeling for opioid receptors, suggesting that many glutamatergic terminals in the mNTS do not contain opioid receptors (7,23).

Since microinjections of naloxone and naloxonazine alone into the mNTS did not exert any cardiovascular effects, it is unlikely that endomorphins play a role in the regulation of cardiovascular function under normal circumstances. However, opioidergic mechanisms may come into play in yet unidentified situations which affect cardiovascular function. For example, opioidergic mechanisms may be activated during stressful situations which elicit pressor and tachycardic responses. Under these conditions, depressor and bradycardic responses elicited by endomorphin in the mNTS may mitigate stress-induced pressor and tachycardic responses. Inhibition of baroreflex in such situations would also be useful because fully functional baroreflex would impede compensatory cardiovascular adjustments to stressful stimuli.

Acknowledgments

This work was supported in part by N.I.H. grants HL024347 and HL076248 awarded to Dr Hreday N. Sapru.

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